DNA analysis workshop

#1 Extract DNA

A) With a DNA-extraction kit

- recommended for samples with strong cell walls - fungi, most plants, seeds, cyanobacteria, etc.
- follow the protocol provided with the kit (this is specific for the organism you want to use)
- this will usually yield much better quality & quantity of DNA than necessary

B) With a DIY protocol

- for soft samples - mammalian cells, many fruits, ground up plant tissue, etc.

#1 mush up the sample in a bag or other container (¼ banana, 3-5 berries, etc.)
#2 solve a small spoon of salt into ½ cup of hot water → add 3-5 big spoons to sample → mix for 1 min
#3 add a small spoonful of dish-washing soap → mix for 1 min
→ you can try the mush directly for the PCR (it's very dirty but often it works)
#4 run the mush through coffee-filter
→ you can try the filtrate directly for the PCR (it's not really pure but often it works)
#5 slowly pour 2-3 big spoonful of ice-cold isopropyl-alcohol/isopropanol down the side of the container
#6 wait ~8 min and observe the DNA forming cloudy clumps → remove some with a big pipette into 'eppi'
#7 centrifuge for 20 sec, remove liquid, wash: add 1 ml water, centrifuge, remove water, mix in 50µl water
→ use ~2 µl of this solution for the PCR

C) Without any protocol

- some samples can be smeared directly into the PCR-tube (just try and you will see…)
- use veeeery little, so little that you can almost not see anything on the tip of the pipette
- the PCR program should then start with 10 min at 95 °C to boil and break the sample

#2 Prepare Overlord-Mastermix

- this should be enough for 9 samples with 10 µl each (technically 10 but calculate with a bit extra)
- divide the Overlord-Mastermix into small PCR-eppis and add your samples to each of them
- number the tubes and document in your labbook/notes

Master-Mix (5x)	20 µl
Primer #1	10 µl
Primer #1	10 µl
Loading buffer (6x)	17 µl
Water	43 µl
End-Volume	100 µl

#3 Run the PCR

- place the PCR-eppis into the PCR-machine and run the following program

	Temp	**Time**	**# of cycles**
Initial Denaturation	94°C	10 min	
Denaturation	94°C	30 sec	
Primer Annealing	55°C	30 sec	35
Extension	72°C	30 sec	
Final Extension	72°C	5 min	

#4 Gel-Electrophoresis

Prepare the Agarose-Gel

- mix 0.5 g Agarose in 50 ml 1 x TBE buffer (dilute from 10x)
- heat in microwave 30 sec full, mix, heat again in short intervals until the solution is clear
- let it cool down until you can touch it with your hand
- add 2.5 µl DNA stain green
- pour into gel-chamber (For BentoLab: make sure the rubbers are in place)
- insert comb for 12 wells or more

Prepare TBE running buffer

- prepare 100 ml 1 x TBE buffer (dilute from 10x)
- add 2.5 µl DNA stain green
- remove the rubbers (only for BentoLab) + comb from the gel-chamber
- use the TBE running buffer to cover the gel (until "max"-marking)
- 100 ml will be enough for 2-3 gels

Load the ladder & samples

- the gel should be completely covered in buffer
- gently pipette 10 µl of the DNA-ladder (premixed with loading buffer and dye) into the first and last well of the gel
- gently pipette 10 µl of the PCR-sample (all of it) into the other wells (document positions)
- if there is some solid or slimy something in the sample, don't pipette it in

Run the gel

- close the box
- run at 50 V for 40 min
- after observation you might want to run again for 10-20 minutes

Observe the result

- remove the lid
- gently place the gel-chamber onto the illuminator
- place the observation-box on top
- switch on the light and look through the hole
- take a picture